

# Mitotane treatment in patients with metastatic testicular Leydig cell tumor associated with severe androgen excess

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## 22 ABSTRACT

23 Mitotane (o,p'DDD) is established in the adjuvant and advanced stage treatment of adrenocortical  
24 carcinoma and counteracts both tumour growth and tumour-related steroid production. Both the  
25 adrenal glands and the gonads are steroidogenically active organs and share a common embryogenic  
26 origin. Here we describe the effects of mitotane in two patients with metastatic Leydig cell tumour  
27 (LCT) of the testes and associated severe androgen excess (serum testosterone 93 and 88 nmol/l,  
28 respectively; male reference range 7-27 nmol/L). Both men suffered from severe restlessness,  
29 insomnia and irritability, which they described as intolerable and disrupting normal life activities.  
30 Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) confirmed excess  
31 androgen production and revealed concurrent overproduction of glucocorticoids and glucocorticoid  
32 precursors, which under physiological conditions are produced only by the adrenal glands but not by  
33 the gonads. In a palliative approach, they were commenced on mitotane, which achieved swift control  
34 of the hormone excess and the debilitating clinical symptoms, restoring normal quality of life. GC-MS  
35 demonstrated normalization of steroid production and decreased 5 $\alpha$ -reductase activity, resulting in  
36 decreased androgen activation, and imaging demonstrated disease stabilization for 4-10 months. In  
37 conclusion, mitotane can be highly effective in controlling steroid excess in metastatic LCTs, with  
38 anti-tumour activity in some cases.

## 39 INTRODUCTION

40 Testicular Leydig cell tumours (LCTs) are rare stromal tumours, comprising 1-3% of all  
41 testicular neoplasms (1, 2). LCTs result in precocious puberty in 10% of affected children due to  
42 excess androgen secretion (3). Affected adult men most commonly present with a painless testicular  
43 mass and significant androgen excess (4) and can also have tumour-related oestrogen excess,  
44 manifesting with gynecomastia in 10-30% of cases (4-6). An estimated 10-15% of testicular LCTs  
45 are malignant (3, 7), although the true proportion remains debated (6, 8). The primary approach to  
46 malignant LCTs is surgical, usually involving orchidectomy, retroperitoneal lymph node dissection  
47 and lifelong surveillance (9). LCT metastases are rare and are detected on average 10 years after  
48 primary surgery (7), but therapeutic options are very limited, with no known role for radiotherapy and  
49 lack of efficacy of cytotoxic chemotherapy (7, 9). Therefore, prognosis for this rare endocrine cancer  
50 is poor, with an approximate median survival of two years (3, 4, 10).

51 During human fetal development, gonads and adrenal glands both derive from the urogenital  
52 ridge and after separation they develop distinct steroidogenic features, with gonadal sex steroid  
53 production and adrenal production of glucocorticoids, mineralocorticoids and adrenal androgen  
54 precursors. Mitotane (o,p'DDD) is routinely used in the treatment of adrenocortical cancer, where it  
55 has been shown to control adrenal steroid excess and, to a degree, tumour proliferation (11). Mitotane  
56 also diminishes androgen action by inhibiting 5 $\alpha$ -reductase (12) and hence activation of testosterone  
57 to 5 $\alpha$ -dihydrotestosterone. Thus, we considered mitotane as a potentially useful drug in patients with  
58 metastatic Leydig cell tumour, in particular in patients with tumour-associated androgen excess. Here  
59 we describe the effects of mitotane treatment in two patients with metastatic LCT, leading to a  
60 significant biochemical and clinical amelioration of the signs and symptoms of tumour-related steroid  
61 excess, and in one of them also to radiological stabilization of previously rapid disease progression.

## METHODS

Urinary steroid metabolome profiling at baseline and during mitotane treatment was carried out by gas chromatography-mass spectrometry, utilizing selected-ion-monitoring analysis for identification and quantification of 32 distinct steroid metabolites reflective of 24-h net steroid output, as previously described (13). Serum steroid measurements were carried out in the routine clinical biochemistry setting, using established and validated tandem mass spectrometry (androstenedione, testosterone) and immunoassays (DHEAS, 17 $\beta$ -oestradiol), respectively.

We carried out immunohistochemistry for sterol-O-acyl transferase 1 (SOAT1) as described previously (14), using antibodies against SOAT1 (1:1000; ab39327; Abcam). The intensity of staining was scored as described by Sbiera et al (15).

## CASE REPORTS

**Case 1:** A 51-year-old patient presented with severe restlessness, insomnia, impaired concentration, increased aggressiveness, redness of the face and body hair growth, all gradually developing over the last six months. Fifteen years previously, he had undergone an orchidectomy for LCT and thirteen years later excision of a retroperitoneal mass, confirmed on histology as LCT metastasis. Imaging revealed multiple lesions consistent with liver, lung and retroperitoneal metastases. Immunohistochemistry of a tissue biopsy confirmed vimentin positive, inhibin negative metastatic LCT. Serum testosterone was very high at 93nmol/L (normal male reference range 7-27nmol/L). Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) showed increased androgen metabolite excretion (sum of androsterone and etiocholanolone 101,476 $\mu$ g/24h; adult male reference range <8000 $\mu$ g/24h) as well as increased excretion of DHEA, metabolites of pregnenolone, progesterone, 17-hydroxypregnenolone, 17-hydroxyprogesterone, and cortisol (230  $\mu$ g/24h; normal <130) (**Fig. 1A**). Prognosis was assessed as poor and the patient declined chemotherapy. However, he agreed to the initiation of mitotane treatment in an attempt to improve the clinical signs and symptoms of tumour-related androgen excess that was significantly limiting his quality of life. Mitotane dose was gradually titrated to 3g per day, with concurrent hydrocortisone

88 replacement (20mg tid). Within a few weeks, androgen excretion decreased from 101,476 to 12,827  
89  $\mu\text{g}/24\text{h}$ , with evidence of significant inhibition of 5 $\alpha$ -reductase activity and normalization of other  
90 steroids that were increased at baseline (Table 1). Plasma mitotane concentrations considered  
91 therapeutic (anti-proliferative) in the context of adrenocortical carcinoma (14-20mg/L) (16) were  
92 reached after 5 months of treatment (Suppl. Table 1). Follow-up imaging still showed progressive  
93 disease at two months, but stable disease according to RECIST 1.1 criteria after six months of  
94 mitotane treatment (Suppl. Fig. 1). Alongside the decrease in androgens, the patient reported a  
95 significant improvement of his previously debilitating clinical signs and symptoms. He returned to  
96 full-time work and enjoyed good quality of life. After 10 months of mitotane treatment, he died  
97 suddenly of a suspected myocardial infarction; no post-mortem examination was carried out.

98 **Case 2:** A previously fit-and-well 59-year-old man presented with a right testicular mass and  
99 underwent orchidectomy; histopathology revealed malignant LCT. Three years later he presented with  
100 lower back pain and imaging showed a large retroperitoneal mass, confirmed as disease recurrence by  
101 transcutaneous biopsy. He underwent laparoscopic removal of the mass together with retroperitoneal  
102 lymph node dissection. One year later, follow-up imaging revealed disseminated metastases,  
103 including liver, kidney and peritoneal deposits. He was unwell, with agitation, anxiety and insomnia.  
104 Biochemical work-up showed increased serum testosterone (88.5 nmol/L, normal 7-27), oestradiol  
105 (744 pmol/L, normal <156), androstenedione (7.0 nmol/L, normal 0.8-3.1), and DHEAS (> 27  
106  $\mu\text{mol}/\text{L}$ , normal 0.91-6.76). GC-MS profiling showed increased steroid excretion including androgen  
107 metabolites (69,108  $\mu\text{g}/24\text{h}$ , normal <8000) and cortisol (414  $\mu\text{g}/24\text{h}$ , normal <130) (Fig. 1A). He  
108 rejected chemotherapy and agreed to palliative mitotane treatment with concurrent hydrocortisone  
109 replacement; mitotane was administered employing the high-dose saturation regimen (Day 1 500mg  
110 tds, Day 2 1000mg tds, and from day 3 onwards 1500 mg tds; therapeutic plasma mitotane levels  
111 were reached after 4 months (Suppl. Table 1). Mitotane decreased serum androgen production within  
112 four weeks. Six months after treatment initiation, plasma testosterone had decreased to 29.1 nmol/L  
113 and oestradiol to 177 pmol/L, while androstenedione and DHEAS had normalized. Urinary steroid  
114 profiling 4 months after initiation of mitotane showed a decline in all previously raised steroid

metabolites and decreased 5 $\alpha$ -reductase activity. This was paralleled by significant clinical improvement in signs and symptoms, specifically reduced restlessness, aggressiveness and insomnia. Imaging four months after initiation of mitotane revealed a mixed response, with regression of some previous lesions but emergence of new metastatic deposits in lung and abdomen. The patient passed away 12 months after his second recurrence, i.e. six months after the start of mitotane treatment.

## DISCUSSION

Here we used mitotane, an established drug in adrenocortical carcinoma, in two patients with metastatic testicular LCT associated with severe androgen excess, clinically manifesting with severe restlessness, insomnia, irritability and impaired concentration. Both patients experienced significant improvement in signs and symptoms with mitotane therapy, swift normalization of steroid excess and some stabilization of radiologically quantified tumour load.

In a comprehensive PubMed search (search terms: Leydig cell tumour, malignant Leydig cell tumour, metastatic Leydig cell tumour, mitotane, lysodren, and o,p'DDD) we identified eight cases of LCT treated with mitotane (**Table 2**). Four patients received mitotane as second- or third-line treatment for metastatic LCT for a very short time only (3 days to 8 weeks); none of them showed a biochemical, clinical or radiological response. The remaining four cases received mitotane as first-line treatment for metastatic LCT, with treatment duration varying between 10 weeks and 33 months (**Table 2**). All four patients experienced significant radiological tumour response and reduction in steroid excess during mitotane treatment. Azer and Braunstein (17) used mitotane to treat a patient with metastatic LCT for six months, resulting in a dramatic response with complete remission of multiple pulmonary metastases, which lasted three months prior to relapse. Radiological reduction in tumour load for several months was observed in two cases (18, 19). Abelson et al (20) noted a significant reduction in 17-ketosteroid excretion and clinical improvement in a metastatic LCT patient treated with mitotane for 18 months, while his disseminated metastases progressed. Adding the experience of our cases, mitotane can be considered a worthwhile palliative option in metastatic LCT, particularly when the disease is associated with steroid excess.

During human fetal development, adrenals and gonads both arise from the urogenital ridge and they both develop steroidogenic capacity, albeit with distinct features, i.e. sex steroid synthesis in the gonads and glucocorticoid, mineralocorticoid and androgen precursor synthesis in the adrenal glands. Benign testicular adrenal rest tumours, which are regularly found in men with congenital adrenal hyperplasia, have been shown to display features of both adrenal and gonadal steroidogenesis (21, 22). Using mass spectrometry, we observed that our two LCT patients showed not only androgen excess, but also increased production of glucocorticoid precursors and cortisol, without clinical signs of Cushing's syndrome. Two previous case reports in patients with malignant LCTs have described ectopic production of steroids normally produced by the adrenal cortex, including cortisol and aldosterone (23, 24). In our two cases, both androgen excess and glucocorticoid overproduction responded well to mitotane treatment. Comprehensive steroid metabolome mapping by GC-MS has been used successfully to differentiate malignant from benign adrenocortical tumours (13). It will be useful to test in future studies whether steroid metabolome profiling would also help differentiate benign from malignant LCT and could have a role in follow-up monitoring.

Recent studies have implicated sterol-A-acyl transferase 1 (SOAT1), previously also termed ACAT-1 for Acyl-coenzyme A cholesterol acyltransferases, as a target of mitotane action (14). SOAT1 is located in the endoplasmic reticulum and involved in intracellular esterification of free cholesterol. John Achermann's group has shown that this enzyme operates downstream of SF-1 and is important for regulation of adrenal steroidogenesis (25). A recent study (14) has provided evidence of inhibition of SOAT1 by mitotane in an adrenocortical cell model, by demonstrating an increase in free cholesterol, oxysterols and fatty acids after treatment with mitotane. We had access to formalin-fixed paraffin-embedded tissue from the tumour recurrence in Patient 2 and used it for carrying out immunohistochemistry for SOAT1 (**Fig. 1B**), which demonstrated predominantly high and moderate expression, detected in 60% and 30% of the cells, respectively. Thus, it is likely that both the steroid-ameliorating and anti-proliferative effects of mitotane are mediated by SOAT not only in adrenocortical carcinoma but also in LCT.



Based on our current findings, the use of mitotane in the palliative treatment of metastatic LCTs of the testes appears feasible and useful, with effective control of tumour-related steroid excess and possible beneficial effects on disease progression, a viable treatment option in a rare endocrine cancer that is not responsive to cytotoxic chemotherapy or radiotherapy.

**Declaration of interest:** The authors have nothing to disclose.

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245 **Figure Legend**

246 **Fig. 1:**

247 **Panel A,** Steroid synthesis in the two patients with metastatic testicular Leydig cell tumour as  
248 assessed by mass spectrometry-based 24-h urinary steroid profiling before initiation of mitotane  
249 treatment (log scale; closed circles, patient 1; open triangles, patient 2). Box plots represent medians  
250 and interquartile ranges from a group of 24 healthy male volunteers (age 40-60 years); whiskers  
251 represent the full range.

252 **Panel B,** Immunohistochemical staining for Sterol-O-acyltransferase 1 (SOAT1) using formalin-  
253 fixed paraffin-embedded tissue from the recurrent tumour of patient 2, demonstrating high (60% of  
254 cells) to moderate (30% of cells) expression of SOAT1 in the tumour tissue.

**Table 1: 24-h urine steroid metabolite excretion ( $\mu\text{g}/24\text{h}$ ) in the two patients with metastatic Leydig Cell Tumour before (=baseline) and during mitotane treatment.**

The male reference range is derived from the 24-h urine steroid excretion observed in 24 healthy men aged 40-60 years. The numbers of the steroid metabolites relate to the numbers in Fig. 1A. The total glucocorticoid metabolites were calculated as the sum of metabolites 20, 22-25 and 27-30. n.m., not measured

		Median (min-max) steroid excretion in healthy men (µg/24h)	Baseline	Patient 1					Patient 2		
				Baseline	Mitotane					Baseline	Mitotane
					Month 1	Month 2	Month 4	Month 6	Month 9		Month 4
	Androgen and androgen precursor metabolites										
1	Androsterone	1,684 (477-5,915)	44,744	13,833	11,823	9,790	4,448	4,440	38,092	597	
2	Etiocolonaolone	1,668 (404-3,393)	56,732	30,283	37,617	22,408	9,798	8,387	31,016	2,697	
3	11β-hydroxy-androsterone	609 (131-2,302)	2,066	1,414	2318	301	169	174	13,351	643	
4	Dehydroepiandrosterone (DHEA)	202 (14-3,948)	1,939	1,034	434	294	194	311	47,344	359	
5	16α-hydroxy-DHEA	269 (0-1,492)	4,404	9,179	4,098	3,510	2,533	6,250	23,569	1,126	
6	5-pregnenetriol	181 (38-951)	1,558	2,574	2,484	2,600	1,575	2,483	20,891	1,053	
7	5-pregnenediol	326 (64-801)	19.972	27,844	24,732	25,548	9,545	15,450	168,192	11,304	
	Mineralocorticoids and mineralocorticoid precursor metabolites										
8	Tetrahydro-11-deoxycorticosterone	94 (22-290)	445	195	162	188	29	128	308	22	
9	5α-Tetrahydro-11-deoxycorticosterone	107 (50-360)	62	100	87	52	32	49	74	18	
10	Tetraydrocorticosterone	97 (24-258)	300	154	263	169	41	105	177	18	
11	5α-Tetrahydrocorticosterone	193 (67-1,197)	130	261	489	0	0	0	90	0	
12	3α,5β-Tetrahydroaldosterone	30 (12-64)	n.m.	n.m.	n.m.	n.m.	13	28	293	25	
13	Tetrahydrodeoxycorticosterone	13 (5-36)	n.m.	n.m.	n.m.	n.m.	93	343	216	37	
	Glucocorticoid precursor metabolites										

14	Pregnanediol	157 (32-336)	3,249	1,857	1,474	1,171	455	646	4,832	199
15	3 $\alpha$ ,5 $\alpha$ -17-hydroxy-pregnanolone	14 (6-89)	n.m.	n.m.	n.m.	n.m.	13	18	809	8
16	17-hydroxypregnanolone	133 (41-537)	7,163	1,940	1,589	1,538	817	998	39,306	551
17	Pregnanetriol	576 (243-1,175)	9,562	5,701	4,295	4,366	2,128	2,677	28,349	1,495
18	Pregnanetriolone	13 (5-58)	20	2	0	3	5	0	1,822	10
19	Tetrahydro-11-deoxycortisol	61 (21-159)	594	525	690	492	314	911	116	322
Glucocorticoid metabolites										
20	Cortisol	57 (22-224)	252	735	497	495	399	813	414	201
21	6 $\beta$ -hydroxy-cortisol	114 (63-504)	n.m.	n.m.	n.m.	n.m.	7,657	23,193	393	3,578
22	Tetrahydrocortisol	1,694 (772-4,534)	4,260	9,578	7,936	5,087	2,115	3,001	2,779	1,391
23	5 $\alpha$ -Tetrahydrocortisol	1,408 (229-6,744)	477	344	161	114	37	66	702	40
24	$\alpha$ -cortol	319 (177-1,005)	1,665	2,566	1,880	1,256	524	831	597	286
25	$\beta$ -cortol	513 (255-1,678)	957	378	306	207	108	153	467	60
26	11 $\beta$ -hydroxy-etiocholanolone	315 (23-899)	257	147	91	95	37	50	1092	89
27	Cortisone	92.5 (39-348)	198	400	389	286	309	480	671	102
28	Tetrahydrocortisone	3,333 (1,465-7,597)	3,978	2,391	2,113	1,564	763	1,124	5,597	807
29	$\alpha$ -cortolone	1,228 (605-2,599)	3,892	2,661	2,222	1,166	410	539	1,623	479
30	$\beta$ -cortolone	696 (417-2,075)	1,110	300	303	216	108	213	1130	42
31	11-oxo-etiocholanolone	464 (74-997)	1,059	734	736	868	844	1,196	3,144	267
Total glucocorticoid metabolite excretion		9,66 (5,467-15,426)	16,789	19,353	15,807	10,391	4,773	7,220	13,980	3,408
Steroid ratios indicative of 5 $\alpha$ -reductase:										
Androsterone/Etiochaolanolone		1.13 (0.05-3.00)	0.79	0.46	0.31	0.44	0.45	0.53	1.23	0.22
5 $\alpha$ -tetrahydrocortisol/tetrahydrocortisol		0.92 (0.05-2.27)	0.11	0.04	0.02	0.02	0.02	0.02	0.25	0.03

**Table 1:** Previously reported cases of patients with widespread metastases from testicular Leydig cell carcinoma treated with mitotane, presented in the order of duration of treatment.

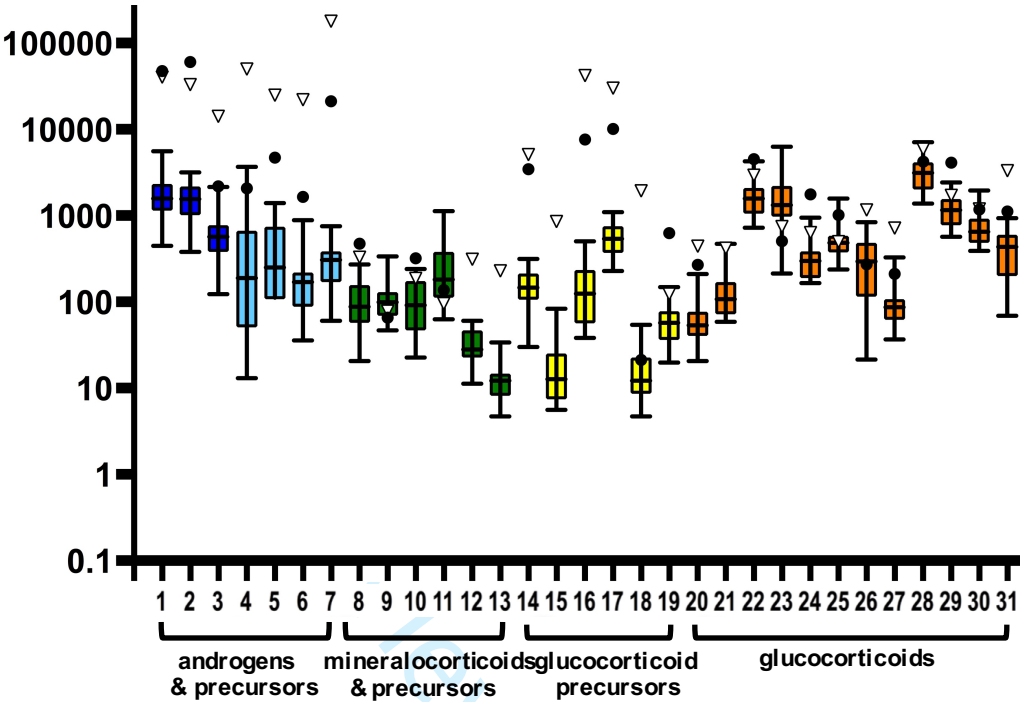
Reference	Patient age (years)	Length of mitotane treatment	Mitotane dose (plasma mitotane levels)	Glucocorticoid replacement	Documented steroid excess	Patient outcome	Additional information whilst on mitotane therapy
<b>Second- to third-line treatment (treatment duration 3 days – 8 weeks)</b>							
Tamoney et al., Cancer 1969 (23)	64	3 days	10g/day (not done)	not reported	Increased urinary 17-ketosteroids, increased urinary estrogen	Died – no effect	First-line radiotherapy (40000 rads cobalt therapy); Died 3 days after commencing mitotane therapy
Grem et al., Cancer 1986 (4)	37	7 weeks	1.5g/day (not done)	not reported	Increased urinary 17-KS, increased serum testosterone, androstenedione, DHEAS	Survived another 5 years on alternative treatment (Lonidamine)	First-line therapy cisplatin; mitotane stopped after 7 weeks due to abdominal discomfort and increasing nausea
Davis et al., Cancer 1981 (24)	61	8 weeks	12g/day (not done)	not reported	Normal urinary 17-KS and 17-OHCS	Died 8 weeks after commencing mitotane	First line therapy cisplatin/vinblastin/bleomycin; Second line therapy cyclophosphamide/doxorubicin/vincristine); Was concurrently on chemotherapy and radiotherapy.

Bertram et al., Cancer 1991 (25)	60	8 weeks	6-12g/day (not done)	Dexamet ha-sone 1mg/day	Normal 17-KS and 17-OHCS; normal serum E1, E2, Aldo	Died after 8 weeks from widespread metastatic disease	2 <sup>nd</sup> line therapy (1 <sup>st</sup> line doxorubicin); No response to mitotane
<b>First line treatment (treatment duration 10 weeks-33 months)</b>							
Schwarzmann et al., 1989 (15)	59	10 weeks	9g/day (not done)	none	Increased serum testosterone, estradiol, aldosterone and cortisol	Died 6 months after commencing mitotane therapy	Reduction in abdominal tumor size and reduction in testosterone and estradiol to normal levels lasting 2 months. Treatment stopped on patient's wish following sudden deterioration and increase in tumor size.
Azer&Braunst ein, Cancer 1981 (14)	63	6 months	4-14g/day during first four weeks, followed by 2.4g/day	Dexamet ha-sone 1 mg/day	Normal urinary 17- KS + 17-OHCS; normal serum aldosterone, testosterone, DHEAS, cortisol	Died after deterioration and continuing metastatic spread of disease. Clinical improvement with mitotane	Complete disappearance of pulmonary metastasis and clinical improvement after 14 weeks on mitotane. 3 months later pulmonary metastasis reappeared, mitotane was stopped and chemotherapy commenced.
Abelson et al., Metabolism 1966 (17)	58	18 months	10g initially, then 4-6g/day (not done)	Dexamet ha-sone 0.375mg twice daily	Increased urinary 17-KS and estrogens	Died after clinical and biochemical improvement with mitotane but radiological progression	Believed to be clinically improving, with reduction in urinary 17- ketosteroids from 1462 to 100 mg/day
Van der Hem et al., J Urol 1992 (16)	56	6 months + 27 months (9 months break in between)	4-10g/day (15-20 mg/L),	Cortisone acetate, no dose recorded	Normal urinary 17OHCS, An, Et, DHEA; normal serum T and DHEAS	Died – metastatic disease stabilized for 18 months on mitotane treatment before disease slowly deteriorated	decrease in retroperitoneal tumor, liver lesions, ascites along with stable disease for 18 months. Once disease deteriorated mitotane dose was escalated to 10g/day with no effect.

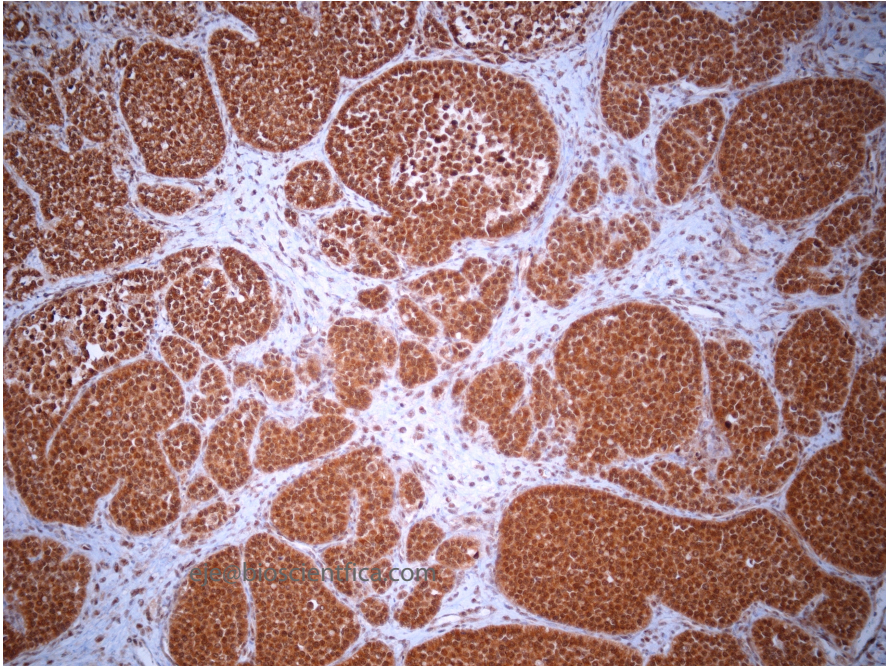


# Urinary steroid profile

Urinary steroid excretion  
( $\mu\text{g}/24\text{h}$ )

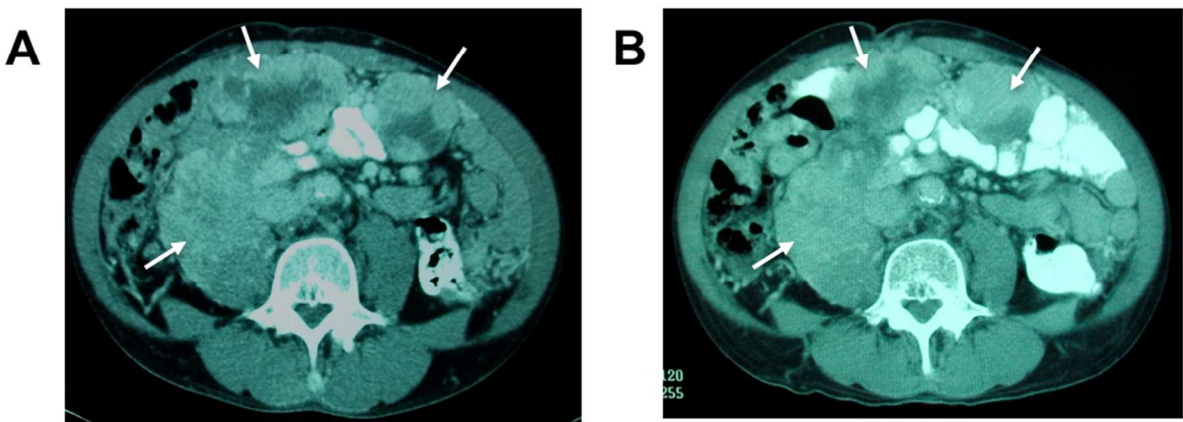


B



**Mitotane treatment in patients with metastatic testicular Leydig cell tumor associated with severe androgen excess**

**Suppl. Fig. 1:** Computed tomography scans taken in Patient 1. **A**, Abdominal CT after two months of mitotane treatment showing metastatic Leydig cell tumor deposits, with heterogeneous omental masses and a large retroperitoneal mass. **B**, Abdominal CT after six months of mitotane treatment, demonstrating stable disease according to RECIST 1.1 criteria.



**Mitotane treatment in patients with metastatic testicular Leydig cell tumor  
associated with severe androgen excess**

**Suppl. Table 1:** Mitotane daily dose and plasma concentrations in the two Leydig cell tumor patients. Therapeutic range for plasma mitotane in adrenal cancer is accepted as 14-20 mg/L

Duration of mitotane treatment (months)	Patient 1		Patient 2	
	Mitotane Dose (g/d)	Plasma Mitotane (mg/L)	Mitotane Dose (g/d)	Plasma Mitotane (mg/L)
1	1.5	4.0	4.5	n.m.
2	3	5.9	4.5	9.1
4	3	9.1	4.5	14.2
5	3	23.5	4.5	n.m.
6	2	17.6		
7	3	9.2		

n.m., not measured.